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Monitoring Imidazoline Derivatives via Functionalized Nano-Potentiometric Platforms in Aqueous Humor and Dosage Forms

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Abstract: The determination of two imidazoline derivatives [oxymetazoline HCl (OXY) and xylometazoline HCl (XYLO)] was described using different potentiometric platforms. The first electrode type was constructed using tetraphenyl borate (TPB) as anionic exchanger with β-cyclodextrins (β-CD) as ionophore forming oxymetazoline-tetraphenyl borate (OXY-TPB) and xylometazoline-tetraphenyl borate (XYLO-TPB), respectively. The second electrode type was prepared by modification of the first type by conjugation with magnetic iron oxide nanoparticles (MNP) forming (OXY-MNP) and (XYLO-MNP). The synthesized electrodes were fully characterized. The effect of magnetic nano-sized particles as a highly dispersible material with β-CDs on the electrode characteristics was investigated and compared against the classical electrodes. The response time, working pH range and selectivity coefficients were studied. The functionalized nano-electrodes (OXY-MNP and (XYLO-MNP) were found to be more sensitive than the classical electrodes with linearity ranges (1×10^{-6}–1×10^{-2} M). The functionalized nano-electrodes were successfully applied for the in-line analysis of OXY and XYLO in pharmaceutical dosage forms and spiked rabbit aqueous humor samples with no prior extraction of treatment. This suggests the future use of these electrodes in clinical studies of both drugs of interest.

Keywords: Oxymetazoline · Xylometazoline · magnetite ferric oxide · sensors · aqueous humor.

1 Introduction

Imidazoline derivatives are sympathomimetic agents which act almost exclusively on α-adrenergic receptors. The imidazoline derivatives act as decongestant and topical hemostatic agents due to their vasoconstricting activities. Imidazoline derivatives are found in different formulations either nasal, for the relief of nasal congestion caused by rhinitis and sinusitis or ophthalmic, or for the temporary relief of minor eye redness and discomfort caused by minor irritants [1]. In July 2020, the FDA approved the use of an ophthalmic formulation of oxymetazoline in adults with acquired blepharoptosis, or ptosis, making it the first FDA-approved medical treatment for this medical condition [2]. Products with imidazoline components are numerous including Oxymetazoline HCl (OXY) 6-tert-butyl-3-(4, 5-dihydro-1H-imidazol-2-ylmethyl)-2,4-dimethylphenol;hydrochloride) and xylometazoline HCl (XYLO) (2-[(4-tert-butyl-2,6-dimethylphenyl)methyl]-4,5-dihydro-1H-imidazole; hydrochloride) as shown in Figure 1.

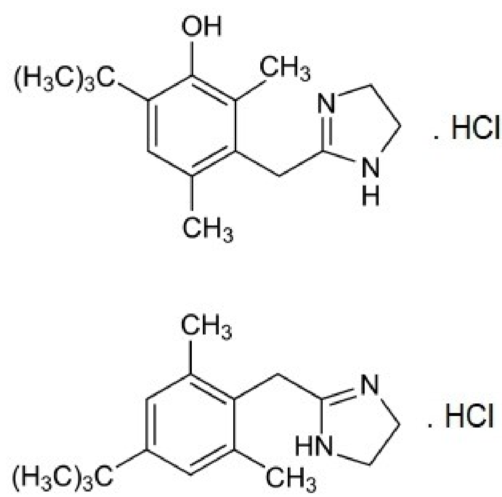

Fig. 1. Chemical structure of (a) oxymetazoline hydrochloride, (b) xylometazoline hydrochloride

Several techniques were reported for the detection OXY and XYLO in pharmaceutical preparations and biological fluids such as spectrophotometric [3] and chromatographic methods [4].

Potentiometric method is one of most widely-used straightforward methods, It is considered simple, eco-friendly, cost effective as well as sensitive portable in-line monitors of small sample with consistent measurements [5]. It has extensively applied in the bioanalytical fields [6], yet few potentiometric methods were reported for oxymethazoline and xylomethazoline detection [7].

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Nowadays, electroanalysis innovation has been expanded by modifying the electrode's surface and increasing the electron transfer rate across it [8]. Nanoparticles have been widely applied to potentiometric sensors based on their unique physicochemical properties, such as large surface area/volume ratio, high conductivity, superior electrocatalytic activity and good mechanical power. Among these nanoparticles, functionalized magnetite nanoparticles have been used as modifiers [9]. Functionalized magnetite nanoparticles with their interesting magnetic properties can be widely distributed in solution and significantly promote the chemical reaction between the functional groups on these particles and the analyte in the sample, which is due to the much lower diffusion layer thickness [10].

Accordingly, this research deals with the development of nano-potentiometric platforms based on dispersion and coating of functionalized magnetic iron oxide nanoparticle (MNP) with ionophore and plasticizer, which leads to the enhancement of in-situ cooperative ion-pairing interaction between the inner solution analyte and the ionophore, resulting in more speed and sensitivity for the measurements of the selected imidazoline derivatives (OXY and XYLO) in pharmaceutical formulations and aqueous humor, in addition to much selectivity in presence of organic and cationic interferents.

Tetraphenyl borate (TPB) ionophore is used as an anionic exchanger with β-cyclodextrins (β-CD) to form two classical electrodes (OXY-TPB) and (XYLO-TPB), respectively. By conjugating magnetic nano-particles (MNP) with the former electrodes, two functionalized nan-potentiometric sensors (OXY-MNP) and (XYLO-MNP) were synthetized and characterized. An investigation was carried out to study the effect of the highly dispersed magnetic nano-sized conjugated with β-cyclodextrins (β-CDs) on the electrode performance characteristics, response time, working pH, detection limit and selectivity. The results were compared against the classical (OXY-TPB) and (XYLO-TPB) electrodes.

2 Experimental

2.1 Equipment

Jenway digital ion analyzer model 3505 (Jenway, UK) with Ag/AgCl double junction reference electrode (Aldrich, USA) was used for potentiometric measurements, while Jenway digital ion analyzer model 3510 (Jenway, UK) was used for the robustness study. Jenway pH glass electrode (Jenway, UK) was used for pH adjustmenst.

2.2 Chemicals and Reagents

All chemicals and reagents used were of analytical reagent grade. High molecular weight polyvinyl chloride (PVC), Tetraphenyl borate (TPB), nitrobenzene (NB), tetrahydrofuran (THF), β-cyclodextrin were obtained from Aldrich-USA, sodium hydroxide scales (NaOH), Hydrochloric acid (HCl), potassium chloride were supplied from El -Nasr company. Oxymethazoline HCl (OXY) and xylomethazoline HCl (XYLO) standards were kindly supplied by Glaxosmithkline (GSK). Nazocrom® nasal spray, labeled to contain 2 % cromolyn sodium and 0.025 % OXY, manufactured by Sigma Pharmaceutical industries, Egypt. Nasotal Compound® nasal drops, labeled to contain 2 % cromolyn sodium and 0.025 % XYLO, manufactured by Amoun Pharmaceutical Co, Egypt.

2.3 Standard Solutions

OXY and XYLO stock solutions (1×10^{-2} M) were prepared in Britton–Robinson buffer and serial dilution of the stock solutions were done using the same buffer for working solutions preparation (10^{-2}–10^{-7} M).

2.4 Procedures

2.4.1 Preparation and Functionalization of Iron Oxide Nanoparticles (Fe_3O_4)

Iron oxide nanoparticles have been synthesized via co-precipitation method. This method based on alkaline co-precipitation of ferric and ferrous salts in aqueous solution [11]. Briefly, two solutions containing Fe II and Fe III at a pre-determined concentration ratio were mixed followed by the addition of a base. Followed by pH adjustment of the solution and addition of a dispersing element to stabilize the particles as shown in Figure 2. After that the nanoparticles were coated with ionophoric polymer β-CD, by mixing equal amounts of MNP and β-CD in NB, the THF solvent was added until complete homogeneity is observed, followed by 30 minutes ultrasonic treatment to ensure that a stable magnetic fluid is

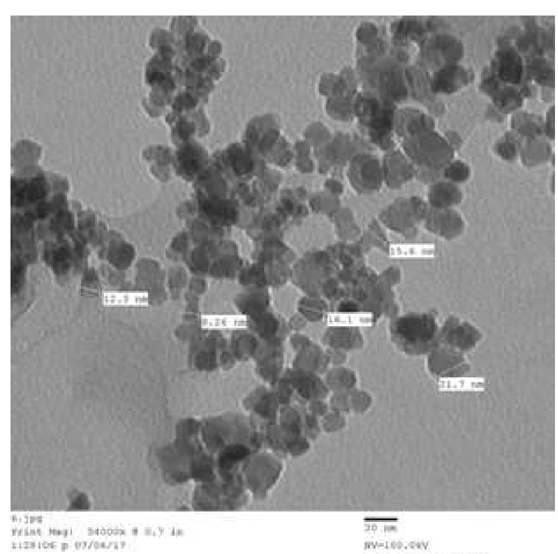

Fig. 2. TEM micrography of magnetite Fe_3O_4 nanoparticles.

formed. At the end, the solvent was evaporated from the magnetic fluid by drying at room temperature.

2.4.2 Fabrication of Potentiometric Platforms

2.4.2.1 Preparation of Classical Electrodes (OXY-TPB and XYLO-TPB)

In a glass petri dish (5-cm) 0.01 g of TPB with 0.04 g of β-CD were incorporated in 0.19 g PVC plasticized with 0.35 g NB, then the mixture was dissolved in 5 ml THF. The evaporation of THF was allowed by drying at room temperature. A thick 0.1 mm master membranes were formed, followed by 0.01 mm disks cut from the master membranes and pasted to the PVC tips using THF and then clipped to the glass electrode bodies.

For OXY-TPB electrode, the internal reference solution was made by mixing two equimolar amounts of OXY and KCl and soaked in OXY solution. The same procedures was done For XYLO electrode but using XYLO solution instead of OXY. The soaking was done for 24 hours before measurement. Ag/AgCl wire (1 mm diameter) acted as an internal reference electrode by immersing it in the internal reference solution.

2.4.2.2 Preparation of Modified Membrane Electrodes (OXY-MNP and XYLO-MNP)

Two electrodes were synthesized by the same procedure mentioned in the former section. In addition, functionalized magnetic iron fluids (20 μL aliquots) were added to the internal reference solution of the electrodes.

2.4.3 Construction of Calibration Plots

All measurements were performed by dipping the prepared electrodes in conjunction with reference glass electrode in 100-mL beakers containing 50 ml of standard solutions under constant stirring until stable potential readings within ± 2 mV was recorded. Calibration graphs were constructed by plotting the potential values (mV) versus logarithm of the concentration. The calibration graphs were constructed by successive addition of 0.01 M solution to 50 mL bi-distilled water to cover the concentration range from 1×10^{-7}–1×10^{-2} M where the corresponding potential of these standard solutions were recorded.

2.4.4 The Effect of pH on the EMF Response of Electrodes

The pH effect on the potential responses of the studied electrodes was tested using two different concentrations (10^{-3} M and 10^{-4} M) of each drug solution over pH range of 3–10 at 1 pH interval. The adjustment of the pH to the desired values was done using either hydrochloric acid or sodium hydroxide. Then the obtained potential was recorded at each pH value.

2.4.5 Estimating Selectivity Coefficients of Electrodes

The potentiometric selectivity coefficients log K_{pot} (primary ion, interfering ion) of different inorganic and organic species were measured using the separate solutions method [12] . The potentials of electrodes in standard OXY/XYLO solutions and aqueous solutions of interfering ions were measured separately at equal concentrations (10^{-3} M). The following equation was sued to calculate the potentiometric selectivity coefficients:

$$\log K^{pot}{}_{AB} = (E_B - E_A)/S$$

Where $K^{pot}{}_{A.B}$ is the potentiometric selectivity coefficient, E_A and E_B are the emf reading of 10^{-3} M solutions of each of the drugs and its interferent, respectively, S is the slope (mV/decade) of the calibration plot.

2.4.6 Analysis of Pharmaceutical Preparations

Five milliliters of each nasal spray (Nazocrom® and Nasotal cpd®) were transferred separately into two 50-mL volumetric flasks, the volume was completed with distilled water to get working solutions of concentration of 25 μg/mL of OXY and XYLO, respectively. The EMF was measured using functionalized nano-electrodes OXY-MNP and XYLO-MNP. Six replicates of each experiment were done. From the recorded potential, the concentration of each of OXY and XYLO were calculated from the corresponding regression equation. The standard addition technique was applied by separately adding different known concentrations of pure standard of OXY and XYLO to the pharmaceutical formulations before proceeding in the mentioned procedure.

2.4.7 Analysis of Spiked Rabbit Aqueous HumorSamples

Two drops of Local anesthetic solution (0.4 % benoxinate HCl) were instilled into the eyes of five albino rabbits. Fresh aqueous humor was immediately removed using 1-ml tuberculin syringe attached to a 26-gauge needle from the anterior chamber of each eye. Afterwards, the ocular surface was washed with isotonic phosphate buffered saline and dried. The procedure was repeated twice daily for five days till the desired amount was collected and then samples were frozen [7c]. At the end of the experiments, the rabbits were scarified.

Half milliliter of the three working solutions (10^{-3} M and 10^{-4} M) of each of standard OXY and XYLO solutions were separately introduced to two sets of 5-ml volumetric flasks and the volume was completed with the collected aqueous humor, and then transferred into test tube and vortex for one minute. The functionalized nano-electrodes OXY-MNP and XYLO-MNP were immersed in these solutions, where the EMF was measured and the concentrations of OXY and XYLO were calculated from their corresponding regression equations.

3 Results and Discussion

The use of potentiometric platforms is highly recommended as an eco-friendly technique due to the reduction of the amount of waste of the analytical methods, the in-line sample determination, eliminating treatment steps and reducing the use of hazardous solvents. All these goals align with the concept of green analytical chemistry (GAC) [13].

Magnetite Fe_3O_4 nanoparticles (MNP) are one of the most commonly used nanoparticles in many fields. MNP acquire several characters which suggests better kinetics for analyte adsorption such as absence of internal diffusion resistance, small size and low volume to surface area ratio [14]. In addition, the introduction of β-CD to the surface of MNP enhances the dispersibility of MNP in the analyte solution and thus maximum surface area and adsorption capacity are achieved [15].

3.1 Membrane Optimization and Composition

OXY and XYLO are organic compounds with imidazoline ring, so they have a cationic exchange capacity. Figure 1. Loading a PVC membrane with anionic salts (TPB) facilitates the selective transfer of this cationic drugs into the membrane phase leaving chloride (co-ion) in an aqueous phase. The bulk of the two phases should be electro-neutral, therefore utilizing lipophilic cation exchangers in the membrane phase would considerably limit the extraction of co-ions providing a typical Nernstian response [16]. Charge separation occurs only in the vicinity of membrane sensors where lipophilic imidazoline-derivatives (OXY & XYLO) cations will be in the organic phase while hydrophilic chloride anions will be in the aqueous phase. This interfacial charge separation generates a phase boundary potential.

The electrodes were soaking first in 1×10^{-4} M of OXY/XYLO solutions as a conditioning step to allow replacement of counter ion (Cl^-) with the target ion.

3.2 Performance Characteristics of the Proposed Electrodes

The electrochemical performance characteristics of the OXY and XYLO classical and functionalized nano-electrodes were investigated according to the IUPAC [12] as illustrated in Table 1. Over a period of 4 weeks, it was found that the four investigated electrodes showed constant potential readings from day to day, and the calibration slopes showed changes by ±2 mV/decade. The slopes of electrodes (OXY-TPB), (OXY-MNP), (XYLO-TPB) and (XYLO-MNP) were 52.3, 55.8, 50.7 and 56.5 mV/decade, respectively. The calibration plots of the four electrodes are shown in Figure 3. Their detection limits were calculated according to the IUPAC definition [12].

Hussein et al. discussed the importance of the iron oxide nanoparticles in the electronic conductivity [17], The nano-size of the iron oxide will positively affect the electronic, mechanical and electric properties. By adding the MNP to the internal reference solution of the electrodes, rapid and symmetric dispersion of the magnetic particles occurred [10]. By applying a constant magnetic field, the magnetic nanoparticles aggregate on the inner side of the membrane, which improves the dissolution of the ionophore and the plasticizer adsorbed on the nanoparticles on the surface of the membrane. This in term yields a significant potentiometric response [18]. Accordingly, the use of magnetite nanoparticles in the functionalized nano-electrodes OXY-MNP and XYLO-MNP resulted in more sensitive detection rather that their corresponding classical electrodes with a linearity range

Table 1. Electrochemical response characteristics of the investigated electrodes.

Parameters	OXY-TPB	OXY-MNP	XYLO-TPB	XYLO-MNP
Slope (mV/decade)[a]	52.3	55.8	50.7	56.5
Intercept (mV)	273.3	288.2	298.2	309.8
LOD (mol.L^{-1})[b]	1.1×10^{-5}	3.2×10^{-6}	2.1×10^{-5}	5.2×10^{-6}
Response time (s)	15	25	15	25
Working pH range	5–8	4–8	5–8	4–8
Concentration range (M)	10^{-2}–10^{-5}	10^{-2}–10^{-6}	10^{-2}–10^{-5}	10^{-2}–10^{-6}
Stability (weeks)	4 weeks	4 weeks	4 weeks	4 weeks
Mean	99.53	100.09	98.84	99.74
Recovery (%)±S.D[a]	±1.55	±1.14	±1.95	±1. 67
Correlation coefficient (r)	0.9982	0.9996	0.9972	0.9991
Accuracy[c]	0.261	0.373	0.402	0.215
Repeatability[c]	0.563	0.694	0.447	0.633
Inter-day precision[c]	0.689	0.420	0.511	0.885
Robustness[d]	0.521	0.632	0.411	0.366

[a] Result of five determinations. [b] Limit of detection (measured by interception of the extrapolated arms of calibration plots). [c] Relative standard deviation of nine determinations of 3 concentrations of each drug (10^{-2}, 10^{-3} and 10^{-4} M). [d] Jenway digital ion analyzer model 3510 (Jenway, UK) with Ag/AgCl double junction reference electrode (Aldrich, USA) was used for potential measurements.

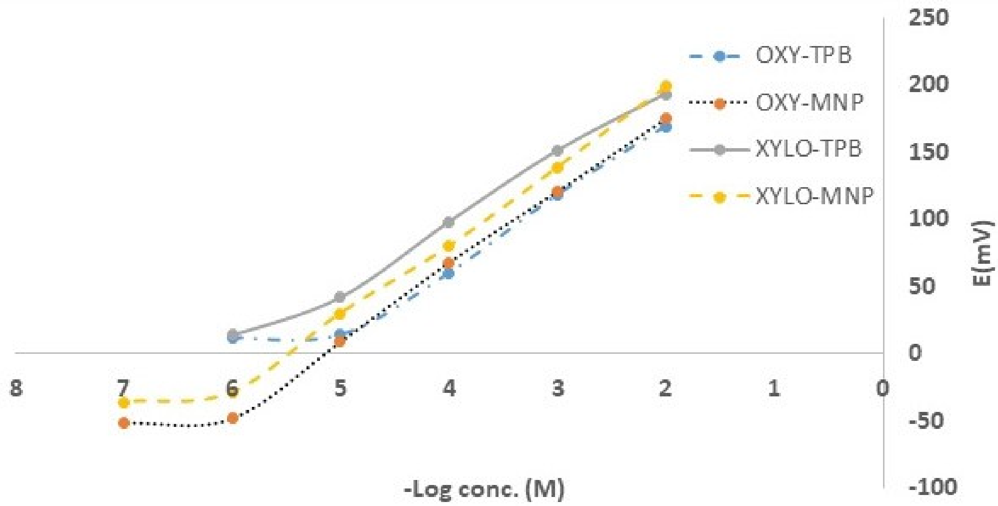

Fig. 3. Profile of the potential in mV to log concentration of OXY/XYLO using the proposed electrodes

(1×10^{-6}–1×10^{-2} M), while the linearity of the classical sensors OXY-TPB and XYLO-TPB were found to be (1×10^{-5}–1×10^{-2} M). Besides, the low limit of detection (LOD) for the functionalized nano-electrodes OXY-MNP and XYLO-MNP were found to be lower than previous reported electrodes for the analysis of OXY and XYLO in different matrices as shown in Table 2.

3.3 Response Time and Selectivity of the Proposed Sensors

Response time is the average required time for the proposed electrodes to reach a potential ±2 mV of equilibrium value upon increasing drugs concentration by 10-fold. It was around 15 seconds for the classical electrodes, while the electrodes with functionalized nanoparticles reached 25 seconds which facilitates the in-line measurements of samples. That could be attributed to the process of nanoparticles aggregation on the inner side of the membrane which resulted in more stable readings.

The selectivity coefficients (K) of the proposed electrodes were calculated in Table 3. It was observed that the functionalized nano-electrodes OXY-MNP and XYLO-MNP exhibit higher selectivity, rather than the classic ones, towards organic and inorganic interferents that may exists in pharmaceutical dosage forms and/or aqueous humor.

The specificity of the proposed sensors were evaluated in presence of different and organic species that can be present as pharmaceutical additives or that can be found in aqueous humor. None of the investigated species interfere as the values of the selectivity coefficients (K) are very small. The inorganic species owing to the difference in the ionic size, their mobility and permeability when compared to imidazoline derivatives. In addition, the proposed sensors showed high selectivity in presence of cromolyn sodium which is the second active

Table 2. Comparison of some electrochemical responses of the proposed electrodes versus reported ones for OXY and XYLO in different matrices.

	Ion-pair	Slope (mV/decade)	LOD (M)	Application	Reference
OXY	Proposed OXY-MNP	55.8	3.2×10^{-6}	Pharmaceutical preparation and aqueous humor	Synthesized electrode
	OXY-phosphotungstic acid	58	1.95×10^{-5}	Pharmaceutical preparation only	7[a]
	OXY-phosphotungstic acid	56.64	5×10^{-4}	Pharmaceutical preparation only	7[b]
	OXY-tetraphenyl borate	59.5	5.0×10^{-6}	Pharmaceutical preparation only	7[d]
XYLO	Proposed XYLO-MNP	56.5	5.2×10^{-6}	Pharmaceutical preparation and aqueous humor	Synthesized electrode
	XYLO-Phosphomolybdic acid	49.78	10^{-4}	Pharmaceutical preparation and aqueous humor	7[c]
	XYLO-Phosphomolybdic acid-β CD	56.21	10^{-5}	Pharmaceutical preparation and aqueous humor	7[c]

Table 3. Potentiometric selectivity coefficients (k) of the four proposed electrodes calculated by separate selectivity method.

Interferent[a]	Selectivity coefficient[b] OXY-TPB	OXY-MNP	XYLO-TPB	XYLO-MNP
Cromolyn sodium	6.90×10^{-3}	5.34×10^{-3}	5.85×10^{-3}	4.43×10^{-3}
Sodium	6.24×10^{-2}	3.56×10^{-3}	4.32×10^{-2}	2.13×10^{-3}
Potassium	4.58×10^{-2}	3.125×10^{-3}	3.77×10^{-2}	2.01×10^{-3}
Calcium	4.07×10^{-2}	2.34×10^{-3}	1.66×10^{-2}	1.08×10^{-3}
Magnesium	2.26×10^{-2}	2.11×10^{-3}	1.59×10^{-2}	1.03×10^{-3}
Cobalt	2.58×10^{-2}	2.08×10^{-2}	1.38×10^{-2}	1.22×10^{-2}
Nickel	1.90×10^{-2}	2.54×10^{-2}	1.15×10^{-2}	1.25×10^{-2}
Zinc	3.98×10^{-2}	2.07×10^{-2}	1.56×10^{-2}	1.06×10^{-2}
Alanine	2.17×10^{-2}	2.39×10^{-3}	1.15×10^{-2}	1.33×10^{-3}
Lysine	9.40×10^{-3}	1.13×10^{-3}	4.87×10^{-3}	4.65×10^{-3}
Glutamine	8.60×10^{-3}	1.68×10^{-3}	5.10×10^{-3}	5.09×10^{-3}

[a] Aqueous solutions of 1×10^{-3} M were used. [b] Each value is the average of three determinations-

ingredient found in the pharmaceutical dosage forms that is attributed to its anionic nature.

3.4 The Effect of pH on the EMF Responses

The responses of the classical OXY and XYLO electrodes were fairly constant over the pH range 4–7, while the functionalized nano-electrodes OXY-MNP and XYLO-MNP showed wider working pH range at (4–8), as shown in Figure 4, which extends the application of the functionalized nano-electrodes to more samples. At pH above 8, the potential started to decline which can be justified by the formation of the basic form of imidazoline in the solution and the ionization of hydroxyl group $[OH]^-$ in highly alkaline media. The working pH of the functionalized nano-electrodes OXY-MNP and XYLO-MNP were suitable for OXY and XYLO detection in aqueous humor with pH equals to 7.5.

3.5 Validation Sheet

The validation parameters for the proposed electrodes were applied as per ICH guidelines [19] including linearity, range, low limit of detection (LOD), accuracy, precision and robustness. Linearity was checked by plotting the -log concentrations as independent variables versus the measured EMF as dependent variables and applying the best fitting straight line where the correlation coefficients for all electrodes were found to be more than 0.997. The functionalized nano-electrodes OXY-MNP and XYLO-MNP showed a wider concentration range (1×10^{-6}–1×10^{-2} M) than their corresponding classical electrodes (1×10^{-5}–$1\times10^{-}$ M), in addition to a lower LOD for both drugs. The accuracy of the proposed electrodes was assessed by the measuring five blind samples of both drugs and the results were presented as recovery percentage (R%) and standard deviation (SD), as shown in Table 1. The precision study included the triplicate measurements of three concentrations of each drug (10^{-2}, 10^{-3} and 10^{-4} M) which were done on the same day (repeatability) and on three successive days (inter-day precision/within lab reproducibility). The results were expressed as relative standard deviation, and all values were found to be less than 2, as shown in Table 1. Robustness was checked by using a different model of potentiometer for the triplicate measurements of three

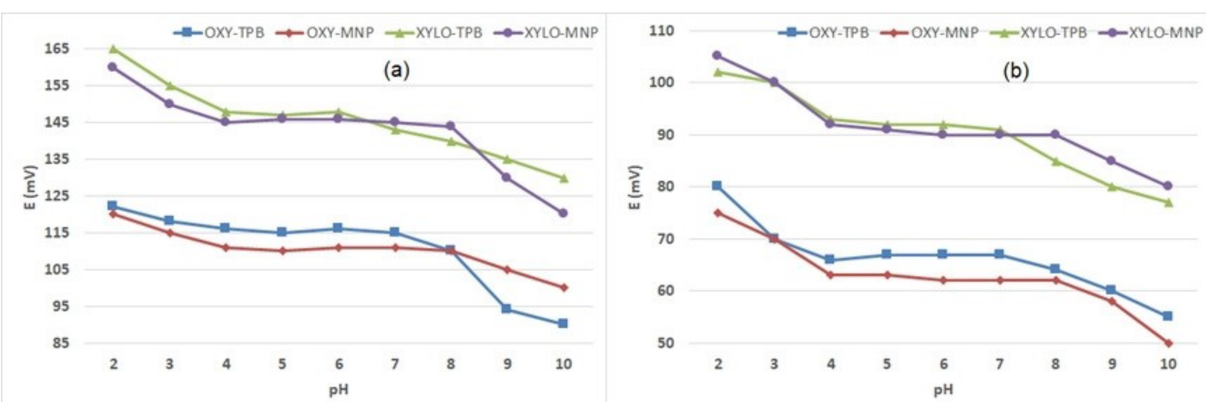

Fig. 4. Effect of pH on the response of the proposed electrodes at concentrations (a) 10^{-3} and (b) 10^{-4} M of OXY and XYLO standard solutions.

concentrations of each drug (10^{-2}, 10^{-3} and 10^{-4} M) and the results were presented as relative standard deviation (RSD) in Table 1.

3.6 Determination of OXY and XYLO in Pharmaceutical Dosage Form

Both drugs, OXY and XYLO, are present as the minor component in the selected pharmaceutical formulations, where their concentrations represent 1/80 of the major component's concentration (cromolyn sodium). Therefore, a sensitive and selective method is required for determination of both OXY and XYLO in presence of cromolyn sodium. Functionalized nano-electrodes OXY-MNP and XYLO-MNP were successfully applied for the analysis of OXY and XYLO in their pharmaceutical formulations, respectively. The standard addition technique proved the applicability of the proposed sensors for the determination of pharmaceutical formulations with no interference of the co-formulated drug or excipients. These data were shown in Table 4.

3.7 Determination of OXY and XYLO in Rabbit Aqueous Humor

The two drugs of interest, OXY and XYLO will be delivered in small quantities through the anterior chamber into the aqueous humor. To simulate this fact, rabbit aqueous humor samples was spiked with low concentrations of each of OXY and XYLO to form solutions of concentrations (10^{-4} and 10^{-5} M). The samples were determined directly using functionalized nano-electrodes OXY-MNP and XYLO-MNP with no prior separation or extraction where stable readings and accurate results were obtained as shown in Table 4. This proved that the proposed functionalized nano-electrodes can be applied to in vitro studies.

Table 4. Analysis of pharmaceutical dosage forms and spiked rabbit aqueous humor samples using the functionalized nano-electrodes (OXY-MNP and XYLO-MNP)

	OXY	XYLO
Nazocrom® nasal spray	102.15 ± 1.23	
Standard addition	99.63 ± 0.65	
Nasotal Compound® nasal drops		99.88 ± 1.63
Standard addition		100.25 ± 0.96
Spiked rabbit aqueous humor		
10^{-4} M	97.66 ± 1.56	101.36 ± 1.93
10^{-5} M	98.14 ± 1.86	98.99 ± 1.49

* The values represent mean recovery percentages ± standard deviations of six readings.

3.8 Statistical Analysis

Statistical analysis of the results using Student's t-test, standard error of means and F-tests revealed no significant differences between the proposed method performance and that of the official methods, as shown in Table 5.

4 Conclusion

The use of potentiometric platforms is highly recommended as it align with the concept of green analytical chemistry; small amount of waste, in-line sample determination and reducing the use of hazardous solvents. The potentiometric sensors are simple and low-cost, don not need sample pretreatment and expensive instruments. The modification of the classical electrodes, through the incorporation of functionalized magnetite ferric oxide nanoparticles in the inner solution produced more sensitive and selective electrodes in comparison to the proposed classical electrodes in our study or the previously reported ones. The functionalized nano-electrodes could be applied for the analysis of OXY and XYLO dosage forms and aqueous humor, with no need for prior sample treatment, and with high selectivity towards inorganic and organic interferents. The future importance of the imidazoline – class was revealed in 2020 where oxymetazoline was the FDA-approved ophthalmic formulation in adults for acquired blepharoptosis, or ptosis. Therefore, further modification of potentiometric sensors can be applied using different nanoparticles.

Table 5. Statistical comparison of the results obtained by the proposed sensors and the official method on pure form

Items	OXY-official method[a]	OXY-TPB	OXY-MNP	XYLO-official method[a]	XYLO-TPB	XYLO-MNP
Mean	100.17	99.95	100.09	100.35	99.84	99.74
± SD	1.19	1.37	1.002	1.49	1.91	1.45
Variance	1.428	1.887	1.004	2.226	3.648	2.102
N	5	4	5	5	4	5
SEM	0.5347	0.6869	0.4480	0.6676	0.9525	0.6504
Student's *t*-test (2.306)[b]		0.267	0.1204 (2.364)[b]		1.343	0.657
F value (6.3882)[b]		1.320	1.424 (6.5914)[b]		1.628	1.053

[a] BP method. [b] Figures between parentheses represent the corresponding tabulated values of t and F at P = 0.05.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request

References

[1] S. C. Sweetman, Thirty-sixth ed., Pharmaceutical Press, London, **2009**.
[2] Vertical Pharmaceuticals, LLC, an Osmotica Company, Announces Acceptance of New Drug Application by FDA for RVL-1201 (Oxymetazoline Hydrochloride Ophthalmic Solution, 0.1%) for Acquired Blepharoptosis (Droopy Eyelid) **2019**.
[3] a) H. M. Lotfy, S. S. Saleh, N. Y. Hassan, S. M. Elgizawy, *Anal Chem Indian J* **2013**, *13*, 152–160; b) O. Abdel-Aziz, A. El-Kosasy, N. Magdy, N. El Zahar, *Spectrochim. Acta Part A* **2014**, *131*, 59–66; c) N. Theia'a, N. M. Mohammed, P. A. Abdul-Jabar, *Eur. Chem. Bull.* **2015**, *4*, 372–377.
[4] a) D. Agbaba, Z. Milojevic, S. Eric, M. Aleksic, G. Markovic, M. Solujic, *J. Planar Chromatogr.–Mod. TLC* **2001**, *14*, 322–325; b) S. Sudsakorn, L. Kaplan, D. A. Williams, *J. Pharm. Biomed. Anal.* **2006**, *40*, 1273–1280; c) G. Golubitskii, E. Basova, V. Ivanov, *J. Anal. Chem.* **2008**, *63*, 875–880; d) G. A. Khan, R. Lindberg, R. Grabic, J. Fick, *J. Pharm. Biomed. Anal.* **2012**, *66*, 24–32; e) M. Fathy, S. Abo El Abass Mohamed, H. Elmansi, F. Belal, *J. Chromatogr. Sci.* **2016**, *55*, 14–22.
[5] M. K. Abd El-Rahman, H. E. Zaazaa, N. B. El-Din, A. A. Moustafa, *ACS Sustainable Chem. Eng.* **2016**, *4*, 3122–3132.
[6] a) B. Zhang, B. Liu, G. Chen, D. Tang, *Biosens. Bioelectron.* **2014**, *53*, 465–471; b) Q. Zhou, D. Tang, *Biochem. Eng. J.* **2018**, *140*, 9–16.
[7] a) Y. Issa, S. Zayed, *Anal. Sci.* **2004**, *20*, 297–300; b) M. A. Ali, *ANJS* **2012**, *15*, 80–87; c) M. Nebsen, G. M. Elsayed, M. Abdelkawy, S. Zaki, *Anal. Bioanal. Chem.* **2013**, *5*, 368–380; d) M. S. Rizk, E. M. Hussien, R. T. El-Eryan, A. M. Daoud, *Eur. J. Chem.* **2019**, *10*, 273–280.
[8] a) V. K. Gupta, B. Sethi, R. Sharma, S. Agarwal, A. Bharti, *J. Mol. Liq.* **2013**, *177*, 114–118; b) H. Karimi-Maleh, F. Tahernejad-Javazmi, N. Atar, M. L. T. Yola, V. K. Gupta, A. A. Ensafi, *Ind. Eng. Chem. Res.* **2015**, *54*, 3634–3639.
[9] A. Rios, M. Zougagh, *TrAC Trends Anal. Chem.* **2016**, *84*, 72–83.
[10] A. M. El-Kosasy, S. M. Tawakkol, M. F. Ayad, A. I. Sheta, *Electroanalysis* **2014**, *26*, 1031–1038.
[11] A. F. Al-Alawy, E. E. Al-Abodi, R. M. Kadhim, *J. Eng.* **2018**, *24*, 60–72.
[12] *IUPAC Analytical Chemistry Division, Commission on Analytical Nomenclature, Pure Appl. Chem* **2000**, *72*, 1851–2082.
[13] A. Gałuszka, Z. Migaszewski, J. Namieśnik, *TrAC Trends Anal. Chem.* **2013**, *50*, 78–84.
[14] A. Ali, M. Z. Hira Zafar, I. ul Haq, A. R. Phull, J. S. Ali, A. Hussain, *Nanotechnol Sci Appl* **2016**, *9*, 49.
[15] a) C. Tudisco, V. Oliveri, M. Cantarella, G. Vecchio, G. G. Condorelli, *Eur. J. Inorg. Chem.* **2012**, *2012*, 5323–5331; b) A. R. Kiasat, S. Nazari, *J. Inclusion Phenom. Macrocyclic Chem.* **2013**, *76*, 363–368.
[16] E. Bakker, E. Pretsch, *Anal. Chem.* **2002**, *74*, 420 A–426 A.
[17] L. A. Hussein, A. M. El-Kosasy, Y. A. Trabik, *RSC Adv.* **2015**, *5*, 37957–37963.
[18] a) D. A. Song, R. N. Liang, R. M. Zhang, J. W. Ding, J. Zhang, W. Qin, *Chin. Chem. Lett.* **2010**, *21*, 1378–1381; b) H. T. Elbalkiny, A. M. Yehia, M. R. Safa'a, Y. S. Elsaharty, *Microchem. J.* **2019**, *145*, 90–95.
[19] Guide, ICH, Q2B, Validation of analytical procedures: Methodology, International Conference on Harmonization, **1997**, Fed. Reg.(62 FR 2643).

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